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Review Article

Mycobacterial proteins—Immune targets for antituberculous subunit vaccine

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Cellular and humoral immunity induced by *Mycobacterium tuberculosis* has led to identification of newer vaccine candidates, but despite this, many questions concerning the protection against tuberculosis remain unanswered. Recent progress in this field has centered on T cell subset responses and cytokines that these cells secrete. There has been a steady progress in identification and characterization of several classes of major mycobacterial proteins which includes secretory/export proteins, cell wall associated proteins, heat shock proteins and cytoplasmic proteins. The protein antigens are now believed to represent the key protective immunity inducing antigens in the bacillus. In this review, various mycobacterial protein antigens of vaccination potential are compared for their efficacy in light of current immunological knowledge.

Tuberculosis caused by *Mycobacterium tuberculosis* is the major cause of morbidity and mortality world-wide. About one third of the world population is infected with *M. tuberculosis* leading to three million deaths annually. Resurgence in the disease trend has been attributed to its association with Acquired Immune Deficiency Syndrome (AIDS) and emergence of multidrug resistant strains. As an approach to development of improved strategies for mycobacterial disease control through vaccination and immunodiagnosis, many researchers have tried to identify individual bacterial component involved in interaction with immune system. In this review, we present a summary to key features of defined proteins, identified as targets to vaccine development.

Current antitubercular vaccination strategy

Bacille calmette guerin (BCG), a live attenuated vaccine derived from *M. bovis* BCG, is the only antituberculous vaccine available. It has many advantages for a vaccine. It can be given at birth or any time thereafter. A single inoculation can produce long lasting sensitization. It is safe and relatively stable. It produces a scar, which can be used for epidemiological surveillance and is inexpensive[1].

Due to these advantages, BCG is being used widespread for control of tuberculosis since, 1928, when it was recommended by the League of Nations[2]. However, BCG has become most controversial of all vaccines due to its low efficacy in some of the recent clinical trials[3,4]. Further, there is a renewed interest and urgency about replacement of BCG especially due to its risk in individuals infected with HIV and emergence of MDR strains[5,6]. This inefficacy and variability of BCG has been attributed to various factors such as methodological flaws, heterogeneity between BCG vaccines used, genetic differences within and between populations, differences in virulence between *M. tuberculosis* strains and interference with or masking of protection by environmental mycobacterial infections[1]. Hence, alternate vaccination strategies are being developed to design more effective and safer vaccine against tuberculosis.

New vaccine designs (Table 1) have been suggested based on—i) insertion of protective antigens into live vectors ; ii) subunit vaccines in conjunction with new synthetic delivery / adjuvant systems; iii) solely peptide based reagents such as multiple antigenic peptide systems (MAPS) and recombinant peptides; and iv) naked plasmid DNA encoding antigenic proteins that confer resistance.

Immune mechanisms in tuberculosis

Characterization of protective mycobacterial antigens and identification of their immunodominant epitopes requires an insight into the protective immune mechanisms involved in resistance to tuberculosis. In the light of recent findings, it seems reasonable that acquired resistance to tuberculosis rests on cell mediated immunity with mononuclear phagocytes and T-lymphocytes being the major factors [19,20]. T-lymphocytes play a dominant role both in protective as well as pathogenic host response to

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mycobacterial infections. Majority of human and murine T cells express αβ T cell receptor [αβTCR] with γδ T cells forming a minority population of usually >5% in blood and lymphoid organs[21]. Number of reports suggest that Th1 subset of CD4+ αβ T cells which are characterized by production of IL-2 and IFN-γ play a pivotal part in protection against tuberculosis[22-25]. Coexistence of TGFβ and IL-12 favours Th1 response[26-28]. Recently, the potential role of CD8+ cytotoxic T cells has also been well documented using culture filtrate antigens of *M. bovis* in inducing protective mechanisms against tuberculosis[29-35]. The type of immune response elicited is also known to be affected by route and dose of immunization as well as influence of costimulatory molecules such as B7-1 and B7-2 (ref. 36,37).

The major breakthrough in the area of foreign antigen recognition by γδ cells came in 1989, when γδ T cell recognition was demonstrated both in human and mice[38-40]. A contradictory report concludes that γδ cells do not directly contribute to protection[41]. Despite conflicting reports, γδ T cells certainly play an important role in providing early cytokines that promote development of Th1 responses and provide an important link between innate and acquired adaptive immunity[42].

A number of reports suggest the importance of mycobacterial proteins as prime target in specific immune recognition as they have easy access to immune systems and are capable of activating specifically CD4+ and CD8+ T lymphocytes involved in protection[43]. Therefore, in this review an update of important mycobacterial protein antigens proposed to be potential vaccine candidates is presented.

Mycobacterial proteins

Identification and classification—The proteins of *Mycobacterium tuberculosis* have been a major research topic almost since the days of discovery of organism by Robert Koch in 1882[44]. Koch pioneered the approach of using components released into the glycerol containing culture medium (Old tuberculin) to monitor immune responses in skin test assays and believed that it might be useful in treatment of tuberculosis[45]. Since then a number of mycobacterial protein antigens have been identified based on biochemical fractionation and immunological screening with monoclonal antibodies and recombinant DNA technology[46,47]. The organisational and physical mapping of *M. tuberculosis* genome and eventual sequence of entire genome is now attainable. The genome comprises 4,411,529 base pairs, containing around 4,000 genes and has high G+C content that is reflected in biased amino acid content of the proteins[48,49]. Besides this, in contrast to other bacteria, mycobacterium has high coding capacity devoted to production of enzymes involved in

Table 1—Potential antituberculous vaccine designs

Type	Delivered molecule	Immune response induced	Ref.
Live vectors			
BCG	Proteins/peptides	CD4/CD8/B*	7
Attenuated Salmonella	Proteins/peptides	CD4/CD8/B	8
Vaccinia virus	Proteins/peptides	CD4/CD8/B	9
Subunit delivery systems			
Liposomes	Proteins/peptides/RNA/ lipids/ cell wall	CD4/CD8/DTH/B	10,11,12
ISCOMS	Proteins/peptides	CD4/CD8/B	13
Microspheres	Proteins/peptides/DNA	CD4/CD8/B	14
cpg motifs**	Proteins/peptides	CD4/CD8/B	15
Chemically defined peptide polymers			
MAPS	Peptides	CD4/B/CD8?)	16
Recombinant	Peptides	CD4/B/(CD8?)	17
Naked plasmid DNA	Protein encoding DNA	CD4/CD8/B	18

*indicates whether carried molecule is known to be able to induce CD4+, CD8+ T cells antibody formation; (B)— bacille calmette guerin (BCG); ISCOMS- immunostimulatory complexes; MAPS- multiple antigenic peptide system; and DTH-delayed type hypersensitivity.
**cpg motifs are cytosine-phosphate-guanine rich sequences present in higher ratio in prokaryotes (16:1) as compared to eukaryotes (50:1). They are potent inducers of innate immunity as well as activators of Th1 and B cell immunity.

lipogenesis and lipolysis. Two new families of glycine rich proteins with repetitive structure that may represent the source of antigenic variations has also been identified[48,49]. By using database comparisons, precise functions to ~84% proteins have been attributed while rest 16% resemble no known proteins and may account for specific mycobacterial functions. Broadly, the mycobacterial proteins can be classified on the basis of functions, in those involved in metabolic pathways; regulation and signal transduction; drug resistance and antigenic proteins.

Metabolic pathways—From the genomic sequence, it is clear that tubercle bacillus has potential to synthesize all the essential amino acids, vitamins and enzyme cofactors, as well as metabolise carbohydrates, hydrocarbons, alcohols, ketones and carboxylic acids[49-51].

Regulation and signal transduction—Extensive regulatory repertoire of *M. tuberculosis* in complex environmental and metabolic stresses are attributed to more than hundred regulatory proteins that are governed genetically by thirteen putative sigma factors[49]. This relative paucity in environmental signal transduction pathways is probably offset by the presence of a family of eukaryotic - like serine / threonine protein kinases (STPKs) which function as a part of phosphorelay system and govern important cellular decisions such as dormancy and cell divisions[52].

Drug resistance—Natural drug resistance is attributed to drug modifying enzymes such as β lactmases and aminoglycosidase acetyl transferases and many potential drug efflux systems such as 14 members of major facilitator family and number of ABC transporters[49,53]. Knowledge of these putative resistance mechanisms will promote better use of existing drugs and facilitate conception of new therapies.

Antigenic proteins - potential vaccine candidates—Attempt to identify and isolate the mycobacterial protein antigens and devise a reference system for the nomenclature using polyclonal antibodies of cell sonicates was done in 1970's (ref. 54, 55). Since then a number of protein antigens have been identified and characterized using molecular biology techniques and mAbs which have been summarised in Table 2. Besides this, there are other mycobacterial proteins which show immunological reactivity but have no identified physiological functions (Table 3).

Secretory proteins—Mycobacteria secrete a number of proteins to the surrounding media[56,57] and these proteins are extremely potent in generation of cellular immune responses and subsequent protection against tuberculous infection[58,59]. A total of 33 proteins have been identified in culture fluid of *M. tuberculosis*, which differ from cytoplasmic proteins in qualitative kinetics and the manner in which these proteins interact with the immune system[46]. The fact that live vaccines are more effective than killed vaccines explains the high immunogenicity of secreted protein based vaccines. T lymphocytes directed toward these proteins may be responsible for initial recognition of the infected macrophages leading to efficient control of infection at an early stage[60-62]. To determine whether a given protein is actively secreted by an organism, formula of localization index or release of isocitrate dehydrogenase (ICD) as marker of autolysis has been worked out[56,62].

The antigen 85 complex comprises the major extracellular protein and is a composite of three different but closely related proteins of approximate molecular mass of 30 kDa[63,64]. Antigen 85 complex proteins are immune targets of cellular and humoral responses in mice, guinea pigs as well as humans[51,58,65-69].

In guinea pigs, strong DTH reaction in response to 30 kDa in antigen presensitized as well as infected animals was observed[70]. Also high level of IFN-γ production and T cell proliferative responses in PBMCs from humans and splenic lymphocytes from mice in response to 30 kDa antigen has been reported[71-73]. Recentlya subunit vaccine containing 30 kDa in conjunction with mild adjuvant was shown to enhance IL-2 and IFN-γ production and conferred protection against caseating disease in guinea pig aerogenic model of tuberculosis[67]. In accordance to these reports, a 30 kDa secretory antigen isolated from the avirulent culture of *M. tuberculosis* in our laboratory is able to induce high level of Th1 responses in preimmunized mice and lead to higher and longer protection than BCG[58,74]. The mechanism of protection involved both MHC class I and II pathways as seen by adoptive transfers in mice where a combination of both CD4+ and CD8+ cells conferred maximum protection[75]. This protein was also evaluated in different strains of mice using various adjuvant formulations such as liposomes, immunostimulating complexes (ISCOMS), dimethyl-dioctadecylammonium bromide (DDA), cytosine-phosphoguanine (cpg), poly-DL-lactide-co-glycolide (PLG) + Lipid A, whereby PLG was found to be ideal

vaccine carrier (unpublished observation). A corresponding protein from the virulent strain has also been isolated. The protein from the virulent culture has been found to be an equally suitable vaccine candidate as it demonstrated high level of immune protection in mice (*in press*). The protective efficacy of corresponding DNA vaccine is being evaluated (unpublished data).

22kDa MPB 70 and 27 kDa protein carrying specific epitopes of *M. tuberculosis* complex have been reported for diagnostic implication[76,77]. Another low molecular mass T cell antigen ESAT-6 has been characterized to be *M. tuberculosis* complex specific and IFN-γ test based on ESAT-6 has been proposed specific for tuberculosis diagnosis[78,79]. ESAT-6 in combination with another *M. tuberculosis* complex specific 28 kDa protein has been proposed to have antigen specific immunostimulatory responses and thus is an important immune target for subunit vaccine development[79,80].

The apa gene encoding 45/47 kDa secretory antigen complex has been cloned and sequenced and has been found to elicit specific immune responses in human[81]. Two other low molecular weight proteins MPT-63 of Mr 16.5 and CFP-28 (both *M. tuberculosis* complex specific antigens), have been proposed for cell mediated immunodiagnosis of tuberculosis[82,83]. Another prominent immunostimulatory proteins of even lower molecular mass of 3-9 kDa have also been characterized[84] out of which ESAT6 has been proposed to be of diagnostic importance. Mapping and characterization of DTH inducing epitopes of MPT64, a major species specific secretory antigen of 23 kDa by use of synthetic

Table 2—Antigens with identified functions

Name	Organism	Subunit size (kDa)	Monoclonal antibodies (mAbs)	Function	Immunological characteristics
DnaK	*M. tuberculosis* BCG *M. leprae*	71 70 70	51A,HAT1,HAT3	Heat shock protein, role in protein folding and translocation	Antibody response in mouse and human, proliferative T cell responses in patients and controls,potential target of autoreactivity /suppress autoimmunity? Protective in mice model of tuberculosis.
GroEL	*M. tuberculosis* BCG *M. leprae*	65 65 65	HAT5, CBA1, H2.16 TB78	Heat shock protein, role in protein folding and translocation	Antibody response in mouse and human, proliferative and cytotoxic T cell responses in patients and controls. recognized by γδ T cells, multiple peptide epitopes mapped. autoreactive responses in rodents and human, protective in mice.
PhoS	*M. tuberculosis*	38	TB71, TB72, HYT28, HBT12, CD38, DI F67.19, HAT2	Role as "binding protein" in phosphate transport potential	*M. tuberculosis* complex specific antibody response in smear positive patients and after BCG vaccination. potential target for subunit vaccine with significant immune responses and protection in mice.
SODA	*M. tuberculosis* *M. leprae*	23 28	F116.5 D2D	Superoxide dismutase	Recognized by monoclonal antibodies.
GroES	*M. tuberculosis* BCG *M. leprae*	12 12 14	SAI 12 CS-01	Heat shock protein, role in protein folding and translocation	Recognized by monoclonal antibodies.induces strong T cell proliferative responses.
	M. tuberculosis	40	HBT10	L-alanine dehydrogenase	Antibody HBT 10 distinguishes BCG and *M. tuberculosis*.

peptides have been pinpointed to 15 residues between aa Gly 173 and Ala 187 (CE15) in guinea pigs prevaccinated with BCG. Recently, initiation and induction of cytotoxic T lymphocytic responses against many culture filtrate antigens of *M. bovis* and *M. tuberculosis* has been reported[29,31]. Certain stress proteins such as 10,65,70 kDa are also detected in culture filtrate and are discussed in following section.

Stress proteins—Stress protein family acts as molecular chaperones mainly mediating protein folding and translocation mechanisms[85,86].

Three members of the stress protein family have been studied extensively and corresponding genes isolated and sequenced-hsp, 70/ DNaK; hsp 60/ GroEL and hsp 10/GroES[85,87-89]. Number of reports suggest hsp 70 to be the immune target in mice and human and is known to be recognised by both CD4+ and CD8+ immune cells[30,90]. The protein possesses a distinct ATPase and autophosphorylating activity that can be modulated by its binding to other proteins and peptides[91]. The special immunogenicity of hsp70 even without use of adjuvant and without prior mycobacterial sensitization has been reported[92].

Hsp 65 has been extensively studied and both T and B cell recognition has been reported to be present in the joints[93]. The protein has been mapped at epitope level for both B and T lymphocyte recognition[94,95]. MHC class II specific CD4+, Th1 cells recognize a single epitope in hsp 65, p 1-20 indicating it to be an important candidate with immunotherapeutic and preventative vaccine potential[96]. Simultaneously, MHC I cytotoxic T cell epitopes on 65 kDa protein has also been mapped[30]. Recently, hsp 65 sequence for identification of rapidly growing mycobacteria has been reported[97]. Immunogenicity studies in human and mice on T cell clones generated to hsp 65 indicate high proliferative responses and IFN-γ and IL-2 secretion indicating protective potential of the protein[98,99]. Recently, it has been demonstrated to induce both CD4+ and CD8+ responses *in vivo* with a requirement of IFN-γ and TNF-α at early stages of infection[35]. However, the protective potential of the protein was underdetermined due to controversial reports on association of autoimmunity and stress of proteins[86,100]. Recently, two independent studies have

Table 3—Antigens with known sequences but without identified physiological functions

Organisms	Subunit size	Monoclonal antibodies	Immunological characteristics
M. intracellulare	43		Serologically active, recognized by T cells
M. leprae	36	F47.9	Specific antibody response in leprosy patients, proliferative and suppressive T cell responses.
M. tuberculosis	35		Serologically active.
M. tuberculosis	30/31	HYT 27	Recognised by monoclonal antibodies cross reactive antibodies in
BCG (MPB44)	30/31	HYT27	leprosy and tuberculous patients, proliferative T cell responses in
BCG (MPB59)	30/31	HYT27	mouse and human; protective in mice and guinea pigs.
M. leprae	30/31		
M. leprae	28		Antibody response in lepromatous leprosy patients.
BCG	23	L24, b4, C24.b1	Antibody responses in humans.
M. tuberculosis	19	TB23, HYT6	Antibody responses in mouse and human, proliferative T cell
M. bovis	19	F29.47, 21.2H3	responses in patients and controls.
M. leprae	18	L5	Antibody response in mouse and human. proliferative T cell responses in patients and contacts
BCG (MPB70)	18		Specific antibody response in *M. bovis* infection.
(MPB80)			
M. leprae	15		Antibody and T cell responses in leprosy patients and healthy contacts.
M. tuberculosis	14		Antibody and proliferative T cell responses in patients species specific antigen.
M. leprae	12		Recognized by antibodies.
M. tuberculosis	10		DTH response in guinea pigs, proliferative responses in human.
M. tuberculosis	6	HYB.76-8	Cell mediated T cell responses, immunodiagnosis, *M. tuberculosis* complex specific.
M. tuberculosis BCG	16.5		*M. tuberculosis* complex specific, cell mediated diagnosis.
M. tuberculosis	28		*M. tuberculosis* complex specific, cell mediated diagnosis.

documented that 65 and 60 kDa hsp in mycobacteria induce resistance in experimental autoimmunity, probably by activating T cells that recognize the homologous hsp sequence present at the site of inflammation[101,102]. The vaccination potential of 65 kDa has been explored in both *M. habana* and *M. tuberculosis* derived hsp 60 and were seen to confer protection against experimental challenges in mice[103,104]. Subcellular localization of 65/60 kDa hsp has been traced to cytoplasm as well as the envelope of mycobacteria using immunoblotting and immunogold ultracytochemistry studies[105]. The 10 kDa GroES homolog is infact found at early stages in culture filtrates[89]. 10 kDa hsp of *M. tuberculosis* proved to be a major T cell immunogen in human and murine studies [106,107]. However, the potential of this antigen for vaccination needs to be explored.

Taken together, these data suggest major heat shock proteins to be potential subunit vaccine candidates. Combitorial vaccine of select CD4+ and CD8+ T cell epitopes could be one mechanism to delete autoimmune side effects of such vaccines if, any.

Proteins associated with the cell wall or membrane—Lipoproteins are generally associated with cell surfaces where the lipid moieties integrate into cell surface and the protein is exposed on the exterior carrying its physiological role[108]. The lipoproteins of Mr 19,26,27 and 38 kDa have been isolated from membrane rich fractions of *M. tuberculosis*[109,110]. Besides their functional aspects, 19 and 38 kDa lipoproteins has been found to be key immunogens[111-113]. The 38 kDa protein stimulates T lymphocytes from immune mice, guinea pigs and humans[70,112,114,115]. The immunodiagnostic potential of recombinant 38 kDa antigen of *M. tuberculosis* exhibits a sensitivity of 72.6% and specificity of 74.6% (Ref. 116). Combination of 38 kDa with 26-28 kDa and 70-71 kDa has also been suggested to be of serodiagnostic value[117]. The major T and B cell epitopes of 19 kDa lipoprotein have also been mapped using linear and conformational analysis[118]. Furthermore, it is of interest that peptides corresponding to the leader sequence of 19 and 38 kDa lipoproteins have been found immunogenic in human[119,115]. Recently, MHC class I binding motifs of 6 mycobacterial proteins 19 and 38 kDa lipoproteins and 10,16, 65 and 70 kDa hsps have been characterized[30]. These dominant cytotoxic T cell epitopes could be important in future analysis of involvement of CD8+ T cells in *M. tuberculosis* infection, pathogenesis and vaccination strategies.

Another antigenic peptide of 23 kDa has been reported from the cell wall peptidoglycan of mycobacteria using chemical treatment method and was found to be immunologically active[120]. Amongst the covalently associated proteins with the cell wall peptidoglycan ,three major antigenic proteins of Mr 71,60,45 have been released from cell wall using trifluoromethanesulfonic acid treatment[121]. The 71 and 45 kDa proteins were found to elicit strong T cell proliferative responses in HPPD+ individuals and tuberculous subjects in early stages of infection[122]. In mice, 71 kDa was found to be most immunoreactive, exhibiting high T cell stimulation with responses skewed towards Th1 subset with high production of IFN-γ and IL-2[123]. Further,a single dose experimental vaccine of 71 kDa using biodegradable and biocompatible polymer based delivery system has been observed to provide long term protection in mice[124]. The mechanism of immunoprotection suggest involvement of both MHC Class I specific CD8+ T cells and MHC class II specific CD4+ immune cells[75].

Cytosolic proteins—Reports on immunogenicity of cytosolic proteins are scattered. A major T cell inducing cytosolic 23 kDa protein antigens from *M. habana* has recently been elucidated to be a superoxide dismutase. The protein induces dose dependent delayed type hypersensitivity reactions in guinea pigs and lymphocyte proliferation in Balb/C mice primed with *M. habana* [125]. The immunogenicity of 65kDa has also been reported and suggest it to be a putative candidate for subunit vaccine[103,105].

Comparative account of major subunit vaccine and their protective potentials have been summarised in Table 4.

However, other factors such as mouse strains, vaccine viability, dose and route of immunization strongly affect the T cell responses and protection and are important criteria to be taken into consideration[36,137].

Future vaccines

DNA or recombinant vaccines have been proposed to be the future approaches for antituberculous vaccine development. DNA vaccination resembles gene therapy to the extent that functional DNA is intentionally introduced into somatic cells. Few mycobacterial proteins have been evaluated for corresponding DNA vaccines and preliminary data are encouraging. Plasmid DNA encoding 65 kDa

Table 4 —Comparison of protective efficacy of various subunit experimental vaccines developed against tuberculosis.

Component	Model	Route of administration*	Adjuvant	Challenge route (dose)	Protective window		Efficacy in comparison to BCG	Ref.
					Difference log 10 CFU	% survival		
Wax D	Mice	id	–	iv (1.0 mg)	–	+ survival	Low	126
Subunit WaxD (PMK 0.2mg)	Mice	id	–	Corneal	Lesion	–	Low	127
PIMs	Mice	sc	FIA	iv (3×10^7)	+	+	Equal	128
PIMs	Mice	sc	Liposomes lipid A	iv (3×10^7)	+	+	Equal	11
Particulate fraction devoid of lipids	Mice	ip	–	iv (2.4×10^7)	–	85%	Equal	129
BCG cell wall	Mice	ip	–	iv	+	–	Equal	130
Cell wall fraction	Mice	sc	FIA	iv (3×10^7)	+	+	Equal	131
Cell wall fraction	Mice	sc	Liposomes	iv(3×10^7)	+	+	Low	10
RNA fraction	Mice	sc	Liposomes/FIA	iv(3×10^7)	+	+	Equal	12
CFP of *M. tuberculosis*	G uinea pigs	sc	FIA	iv	+	–	Comparable	61
CFP of *M. tuberculosis*	Guinea pigs	sc	FIA	Aerosol	+	+	Higher	59
ST–CF of *M. tuberculosis*	Mice	sc	DDA	iv	+	–	Higher	84
30 kDa+ other CFP	Guinea pigs	sc	Syntax (SAF–1)	Aerosol	+	–	Higher	132
ST–CFP	Mice	sc	FIA	iv	+	–	Equivalent (fall at prolonged time)	133
38 kDa lipoprotein	Mice	sc	PLG	iv	+	–	Lesser but higher CMI than FIA	134
65 kDa DNA vaccine	Mice	sc	–	iv	+	–	Higher	104
30 kDa DNA vaccine	Mice	sc	–	iv	+	+	Higher	135
30 kDa (avirulent)	Mice	sc	FIA	iv(3×10^7)	+	95.7	Higher	58
30 kDa (avirulent)	Mice	sc	Liposomes	iv (3×10^7)	+	73.3	Equal	74
30 kDa (virulent)	Mice	sc	PLG	iv(3×10^7)	+		Higher & subtained	Sharma *et al* 1999 (in press)
71 kDa cell wall protein	Mice	sc	FIA	iv(3×10^7)	+	70	Better	123
71 kDa cell wall protein	Mice	sc	PLG	iv(3×10^7)	+	85	better & sustained single dose vaccine	124
hsp65 from *M.habana*	Mice	sc	–	iv	+	+		103
23 kDa SOD from *M. habana*	Mice	sc	–	iv	+	+		125
ATP binding PstS cassette	mice	iv	–	iv	+	+	Better	65
CFP	Guinea pigs/mice	sc	Adjuvant IL–2	iv (10^6 cfu)	+	+	Better	67
30 kDa encoding plasmid	Mice/ guinea pigs	im	–		+	+	Better	67
Plasmid encoding 65 kDa hsp 70, 36kDa and 6 kDa proposed	Mice	im	–	aerosol	+	+	Equivalent	137

*Routes of administration: id—interdermal; Sc—Subcutaneous; ip—intrapertioneal; iv—intravenous; im—intramuscular.

mycobacterial antigen elicited specific cellular and humoral responses and protection in mice which is equivalent to that obtained by vaccinating with live BCG[104]. Similarily, plasmid constructs encoding antigen 85 complex component conferred significant protection against challenge with live *M. tuberculosis* and *M. bovis* BCG in mice[135]. DNA encoding Ag 85 protein could also prevent the onset of caseating

disease in guinea pigs, which is a hallmark of aerogenic challenge in guinea pigs[67]. The genes in *M. tuberculosis* genome encoding proteins to periplasmic ATP bindng casette phosphate receptor (PstS) i.e. Pst-I, Pst S-2 and Pst S-3 have been shown to induce high immunogenicity and protective efficacy against intravenous challenge with *M. tuberculosis* $H_{37}Rv$. Immune responses skewed towards Th1 subset marked by IL-2 and IFN-γ are followed by decreased bacterial load after challenge in presensitized animals[65]. *Ex vivo* transfection approach with a retroviral vector shows that even a single antigen like hsp 70, 65, 36 and 6 kDa are also capable of inducing protection when expressed as a transgene and the induced protection was largely a function of antigenic specific CTL cells[136]. Both viral and murine gene promoters have been found to be effective, but systematic comparisons and optimization of plasmid vectors of DNA vaccination remains to be done. Indeed, special safety issues have to be addressed in addition to efficacy, before that clinical evaluation of DNA vaccines in human can be contemplated.

Thus, we can say that DNA vaccination offers a means of lifelong delivery of protective protein antigens to the immune system. This may also overcome the limitation of using a whole live vaccine such as BCG which has risk of dissemination in immunocompromised host . However, amongst the characterized proteins, still much remains to be done in context with characterization of functional CD4 and CD8 epitopes and their combinations need to be evaluated for ideal subunit vaccine development. Further, the identification and structural analysis of genetically permissive epitopes of *M. tuberculosis* such as p 61-80 of 19 kDa protein and p 331-335, p 441-455 and p 501-515 of hsp 60 may be a useful strategy for the rational design of peptide based vaccines for tuberculosis [138-140].

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